AD_____

Award Number: DAMD17-01-1-0627

TITLE: Is Breast Tissue from Women Who Carry Germ-Line *BRCA1* or *BRCA2* Mutations "Normal"? An Immuno-Histopathological Study

PRINCIPAL INVESTIGATOR: William D. Foulkes, Ph.D.
L. Alpert
J. Deschenes
G. Tremblay

CONTRACTING ORGANIZATION: Sir M. B. Davis-Jewish General Hospital
Montreal, Quebec H3T 1E2 Canada

REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2002	Annual (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE
Is Breast Tissue from Women Who Carry Germ-Line *BRCA1* or *BRCA2* Mutations "Normal"? An Immuno-Histopathological Study

5. FUNDING NUMBERS
DAMD17-01-1-0627

6. AUTHOR(S)
William D. Foulkes, Ph.D., L. Alpert, J. Deschenes, G. Tremblay

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Sir M. B. Davis-Jewish General Hospital
Montreal, Quebec H3T 1E2 Canada

E-Mail: william.foulkes@mcgill.ca

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Background and Hypothesis: *BRCA1/2* mutations confer a substantially elevated risk of breast cancer. It is not known whether breast tissue from *BRCA1/2* mutation carriers is normal of not. We hypothesize that breast tissue in *BRCA1* or *BRCA2* mutation carriers exhibits particular morphological and biological features resulting from BRCA1 or BRCA2 haplo-insufficency or from other additional non-characterized genetic changes, when compared to age-matched non-carriers.

Methods: Forty *BRCA1* or *BRCA2*-related breast cancers and 80 age-matched breast cancers in *BRCA1/2* non-carriers diagnosed in Ashkenazi Jewish women will be analyzed. So far we have examined 510 pathology blocks from 43 women with breast cancer. In order to maintain blinded status, the pathologist does not know how many of these women have *BRCA1* mutations or *BRCA2* mutations. Slides have been cut, mounted, stained and independently reviewed by two pathologists. We plan to evaluate the following biological characteristics: hormonal pathways (estrogen and progesterone receptors, pS2), cell cycle regulation (p27, p53, cyclin D1, cyclin E), proliferation (MIB-1, PCNA), proto-oncogene expression (ERBB2), apoptosis (Bcl-2, caspase3), and androgen receptor.

14. SUBJECT TERMS
breast cancer, immunohistochemistry, *BRCA1*, *BRCA2*

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

Germ-line mutations in *BRCA1* and *BRCA2* genes are the major causes of genetic predisposition to breast cancer. *BRCA1* and *BRCA2*-related breast cancers are characterized by certain pathological features. Increased incidence of medullary type, higher grade, poor differentiation, absence of steroid receptor expression and aneuploidy are all characteristics of *BRCA1*-related breast cancer[1]. *BRCA2*-related breast cancers also tend to be higher –grade tumors than non-hereditary cases. Notably, it has been suggested that *BRCA1* and *BRCA2*-related breast cancer may follow different carcinogenic pathways when compared to sporadic breast cancer[2]. One preliminary study showed a significantly lower expression of the progesterone receptor in the non-neoplastic mammary tissue from a small number of *BRCA1* and *BRCA2* carriers[3]. Interestingly, recent data suggested physiological differences in *BRCA1* and *BRCA2* carriers, such as a reduced period of lactation, when compared with women without mutations[4]. Moreover, contrary to what is observed in the general population, early pregnancy and multiparity are associated with an increased breast cancer risk in *BRCA1* and *BRCA2* mutation carriers[5]. Despite these findings, very little is known about morphological and biological features of non-neoplastic breast tissue in *BRCA1* and *BRCA2* mutation carriers. Here, we plan to study non-neoplastic breast tissue from women identified as being at high risk of developing breast cancer due to the presence of a mutation in one of these genes. This proposal was awarded a Concept grant.

Body of text

Task 1: Collect normal breast tissue and cancers from *BRCA1/2* mutation carriers and controls

The study was considerably delayed by the need for a modification of page 10, paragraph 19, prohibition of use of human anatomical substances (Nov 2000)(USAMRAA).
At the Sir M.B. Davis-Jewish General Hospital, we have collected and are in the process of reviewing 510 pathology blocks from 43 women with breast cancer. In order to maintain blinded status, the pathologist does not know how many of these women have *BRCA1* mutations or *BRCA2* mutations. To increase our potential for recruitment, we have submitted the proposal to the McGill University Health Centre Research Ethics Board (MUHC REB). The Board asked for changes to the consent form. We have submitted these for approval. The amended consent form will be forwarded to the DOD for approval (as it differs slightly from the one approved at the primary site). Once the study is approved at the second site, we expect recruitment to increase considerably. The MUHC REB has also questioned the need for paragraph 18 modification. There is some debate within the REB whether or not this project actually involves human subjects, or rather tissue from human subjects. This debate has further delayed our progress at the second site.

Task 2: Creation of a grid to score the abnormalities noted in normal breast tissue

This has been completed by Dr. Alpert

Task 3: Scoring of abnormalities

This is underway but has not been completed

Task 4: Immunohistochemical analysis of tumours

This is has not be started

Key Research Accomplishments

Nil

Reportable outcomes

1. Publications

No publications have followed from this work as yet. However, obtaining this grant has provided impetus to other, closely related work (please see next section).

2. Other grant support

As a result of this award, we have obtained further funding from the Fonds de la Recherche en Santé du Québec (FRSQ) to collect tissue from *BRCA1* or *BRCA2* mutation carriers. We will collect both normal and cancer tissue. This will allow us to compare the results obtained in this study with those obtained in the FRSQ-funded proposal.

Conclusions

The demonstration of differences in the morphological or biological features of non-neoplastic breast tissue in an ethnically restricted population of *BRCA1* and *BRCA2* mutation carriers will be a crucial step in the understanding of hereditary breast cancer. Moreover, preventive strategies could be influenced by these observations. We have commenced the study, but due to unforeseen circumstances, have not yet completed the work, and as such have no scientific data to report at this time. We have requested a no-cost extension to complete this study.

References

1. Breast Cancer Linkage Consortium. Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. Lancet 1997; 349:1505-10.

2. Tirkkonen M, Johannsson O, Agnarsson BA, *et al.* Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res 1997; 57:1222-7.

3. Kirk JA, Applebee K, Leary J, *et al.* Biological characteristics of the breast in women with a genetic predisposition to breast cancer [Poster]. Am J Hum Genet 1999; 65:A133.

4. Jernström H, Johannsson O, Borg A, Olsson H. Do BRCA1 mutations affect the ability to breast-feed?: significantly shorter length of breast feeding among BRCA1 mutation carriers compared with unaffected relatives. Breast 1998; 7:320-24.

5. Jernström H, Lerman C, Ghadirian P, *et al.* Pregnancy and risk of early breast cancer in carriers of BRCA1 and BRCA2. Lancet 1999;354:1846-50.